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BAYER LEVERKUSEN (GERMANY)

Department for Crop Protection

*Central Research Laboratory of Farbenfabriken Bayer Aktiengesellschaft,
Leverkusen*

A new group of non-phosphorous acaricides*

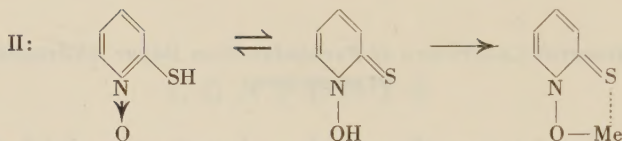
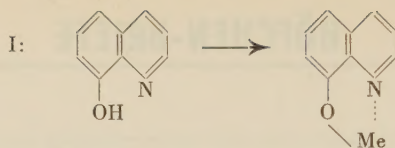
by K. Sasse

The control of spider mites was not a great problem to agriculturists until a few years ago. The damage caused by these pests, compared with that inflicted by other plant parasites, was of secondary importance, and applications of the proved phosphate insecticides kept both spider mites as well as noxious insects well in check. Recently, however, more and more reports are being received from agriculturists and other sources that the products hitherto available are no longer adequate for the increasing infestation density to be effectively suppressed. The main cause of this is twofold; on the one hand, there is the reduction in the population density of natural enemies, and on the other hand the development of resistance which, although varying regionally in its scale and distribution, continues to increase steadily, so that it has become necessary to treat crops infested by spider mites with specific acaricides in combination with applications of insecticides.

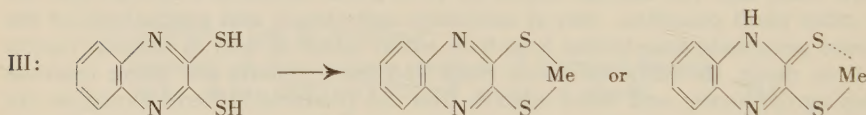
Field observations have revealed that spider mites have succeeded, within a few years, in developing strains that are resistant not only to phosphates but also to non-phosphorous acaricides. Consequently, it is essential that any new acaricide must possess a mechanism of action completely differing from that of products so far used. Our knowledge of the physiological-chemical processes that take place when an insecticide becomes effective in the living organism of plants and insects is still very limited; therefore, it is only in the rarest of cases that a reliable prediction can be made whether a chemical substance, by virtue of its structure, will produce a very definite action in the biological functions. Hence the work of a chemist, engaged on developing pesticides by synthesis, can only be carried out according to strictly empiric conceptions in his efforts to discover basically new active substances.

In our work of developing new fungicides and acaricides, we believed that we could achieve our aim by seeking compounds which are capable of intercepting the metal ions that act as catalysts in many biochemical processes. 8-hydroxyquinoline (I) and pyridine-N-oxide-2-thiol (II) are examples of substances of this kind, already known to have a fungicidal action.

* Dr. R. Wegler largely contributed to the discovery and systematic development of this new group of compounds, through his many valuable suggestions.

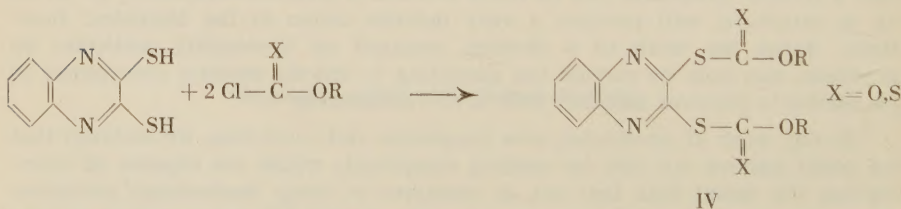


Our studies of different compounds with similar structural properties also included 2,3-dimercapto-quinoxaline (III) ¹).



This compound, however, possesses no really outstanding biological properties either in the free form or in the form of its salts. Thus it was all the more surprising to find that by blocking the SH groups with residues of organic or inorganic acids, compounds with an extremely strong acaricidal and fungicidal action are obtained.²⁾ Development of the carbonic acid derivatives of 2,3-dimercapto-quinoxaline soon proved to be especially worthwhile.

A large number of compounds of type IV were synthesized by the action of chloroformic acid esters or thiocarbonic ester chlorides on 2,3-dimercapto-quinoxaline.³⁾

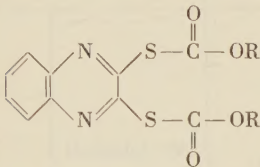
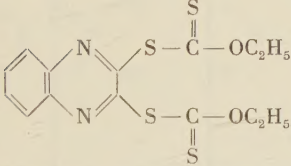


¹) First described by D. C. Morris and A. Furst, J. org. Chem. 21, 470 (1956).

²) Testing of compounds for acaricidal efficacy was carried out by Prof. Dr. G. Unterstenhöfer, and for fungicidal action by Dr. F. Grewe and Dr. H. Kaspers.

It is evident from Table 1 that the acaricidal efficacy of compounds IV rises as the chain length of the aliphatic ester remainder increases from ethyl to butyl resp. isobutyl, while the action of methyl ester approximately corresponds to that of propyl ester.

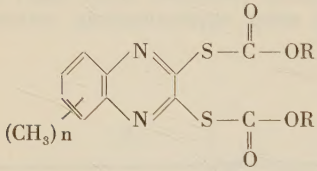
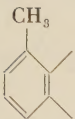
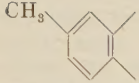
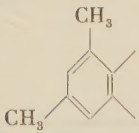
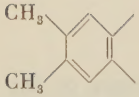
Table 1: Acaricidal action on *T. telarius*

	Melting point	Mortality after 24 hrs:
R = CH ₃	135° (alcohol)	+
R = C ₂ H ₅	99° (alcohol)	—
R = C ₃ H ₇	85° (ligroin)	+
R = iso—C ₃ H ₇	105° (ligroin)	—
R = C ₄ H ₉		+++
R = iso—C ₄ H ₉	65° (ligroin)	+++
R = CH ₂ —CH ₂ Cl	123° (alcohol)	++
R = CH ₂ —CH ₂ —OC ₂ H ₅	124° (acetic ester/ ligroin)	—
R = CH ₂ — 	98° (alcohol)	—
R = — 	82° (alcohol)	++
	138° (benzene/ ligroin)	++

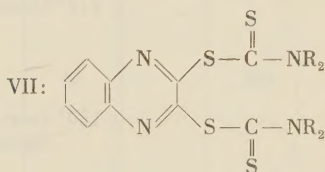
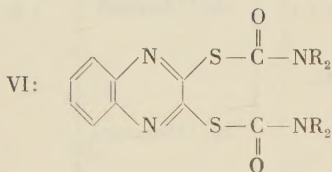
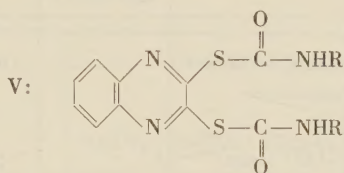
— = < 50%₀ at conc. of 0.2%₀
 + = 50—95%₀ at conc. of 0.2%₀
 ++ = 100%₀ at conc. of 0.2%₀
 +++ = 90—100%₀ at conc. of 0.02%₀
 ++++ = 90—100%₀ at conc. of 0.002%₀

The influence of the methyl group in the benzene nucleus of the quin-oxaline skeleton is surprisingly great; it is noticeable that the compounds with the lowest melting point have the greatest efficacy, probably due to their higher lipide-solubility (Table 2).

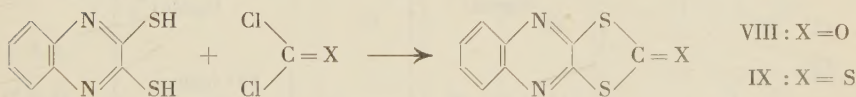
Table 2:

 $(CH_3)_n$	$R = CH_3$ Melting point	Acaricidal action on <i>T. telarius</i> (mortality after 24 hrs.)	$R = C_2H_5$ Melting point	Acaricidal action on <i>T. telarius</i> (mortality after 24 hrs.)
	135° (alcohol)	+	99° (alcohol)	—
			63° (ligroin)	++
	93° (ligroin)	++	105° (ligroin)	+
	—	+++	—	++
	168° (alcohol)	+		

The substitution of the ester groups in compounds IV by amide groups, as for example in V—VII, usually leads to a reduction of the acaricidal efficacy.



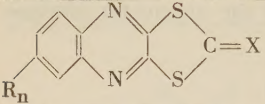
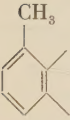
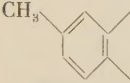
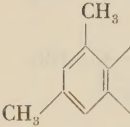
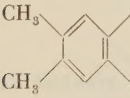
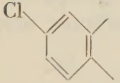
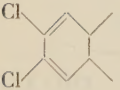
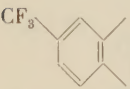
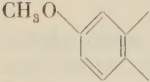
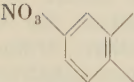
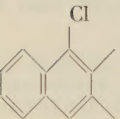
On the other hand, access to the substances that have the greatest acaricidal efficacy in this group is gained by joining the two SH groups of 2,3-dimercaptoquinoxaline to the ring through the remainder of carbonic acid or thiocarbonic acid.³⁾



The test results summarized in Table 3 show that the cyclic dithiocarbonates (VIII) usually have a higher initial toxicity to spider mites than the trithiocarbonates (IX). The efficacy largely depends upon the nature of the substituents in the benzene nucleus of the quinoxaline skeleton; the same efficacy or an even better action is attained especially by a methyl-, trifluoromethyl- or alkoxy-group in 6-position, as compared with the non-substituting basic compounds, whereas carbonamide and sulphonamide groups lead to a practically complete cancellation of the biological properties.

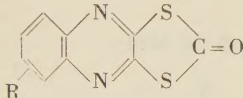
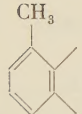
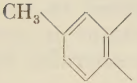
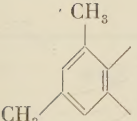
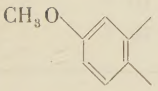
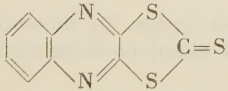
³⁾ Belg. Pat. 580 478; F. Pat. 1229 384; Patents granted also in Spain, Portugal, Israel, Turkey, South Africa, Argentine and Columbia, DAS 1088 965 (1958), Farbenfabriken Bayer Aktiengesellschaft, invented by K. Sasse, R. Wegler and G. Unterstenhöfer.

Table 3:

	X = O Melting point	Acaricidal action on <i>T. telarius</i> (mortality after 24 hrs.)	X = S Melting point	Acaricidal action on <i>T. telarius</i> (mortality after 24 hrs.)
	183° (dioxane)	++++	180° (dioxane)	++
	135° (tetra/ ligroin)	+++	140° (tetra/ ligroin)	—
	172° (benzene)	++++	152° (dioxane)	++
	180° (tetra/ ligroin)	+	167° (benzene)	—
	208° (toluene)	+++	211° (dioxane)	—
	209° (dioxane)	+++	172° (glycol- monome- thylether)	+
	120° (xylene/ ligroin)	—	125° (dioxane/ ligroin)	—
	135° (alcohol)	++++	83° (chloro- form/ ligroin)	+++
	166° (dioxane)	++++	159° (glycol- monome- thylether)	++
			143° (glycol- monome- thylether)	—
	250° (xylene)	—	220° (xylene)	—

A similar gradation is also obtained on comparing the ovicidal properties. (Table 4)

Table 4:

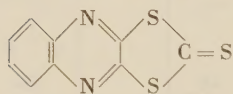
	Ovicidal action on <i>T. telarius</i> (in %) at concentrations of					
	0.02 %	0.01 %	0.005 %	0.002 %	0.001 %	0.0005 %
						
	100	100	46		16	15
	99	90	75	33		
	100	100	100	100	99	93
	70		46			
	100	100	100	100	95	37
	96	54	47			

In large-scale field trials it was found that the difference in acaricidal efficacy between the dithiocarbonates (VIII) and the trithiocarbonates (IX) is not so great as might have been expected from the greenhouse test results. The trithiocarbonates, on account of their better stability to hydrolysis, have a

much longer residual action, so that they were given preference for practical use.

In further biological testing of the quinoxaline derivatives with a strong acaricidal action, it was observed that the same compounds are also extremely effective against the resistant mildew fungi, this action being not only protective but also eradicated⁴). The fungicidal efficacy of the different compounds of this series is generally, although not always, dependent upon the same structural relations as for the effect against spider mites. The field trials, although not completed, so far indicate that 6-methyl-quinoxaline-2,3-dithiol-carbonate (Ss 2074) is the most promising substance.

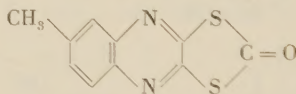
1. Physical properties of Ss 1451 and Ss 2074



Ss 1451

yellow to orange-brown
crystals

Melting pt. 180° C



Ss 2074

light-yellow, almost colour-
less crystals

Melting pt. 172° C

Both compounds are thermally stable, and can be sublimed in vacuum without decomposing. The compounds are practically non-volatile in normal temperature conditions. The finding that they produce a considerable action also in the gas phase is all the more astounding.

Vapour pressure (in mm Hg) ⁵⁾ at temp. (in ° C) of	Ss 1451	Ss 2074
20 °	1×10^{-7}	2×10^{-7}
100 °	1×10^{-3}	3×10^{-3}

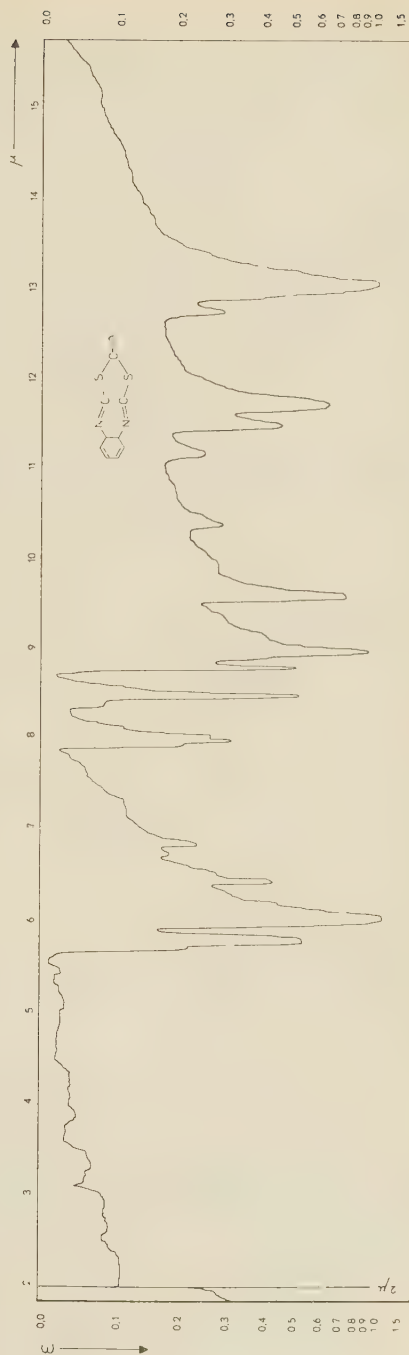
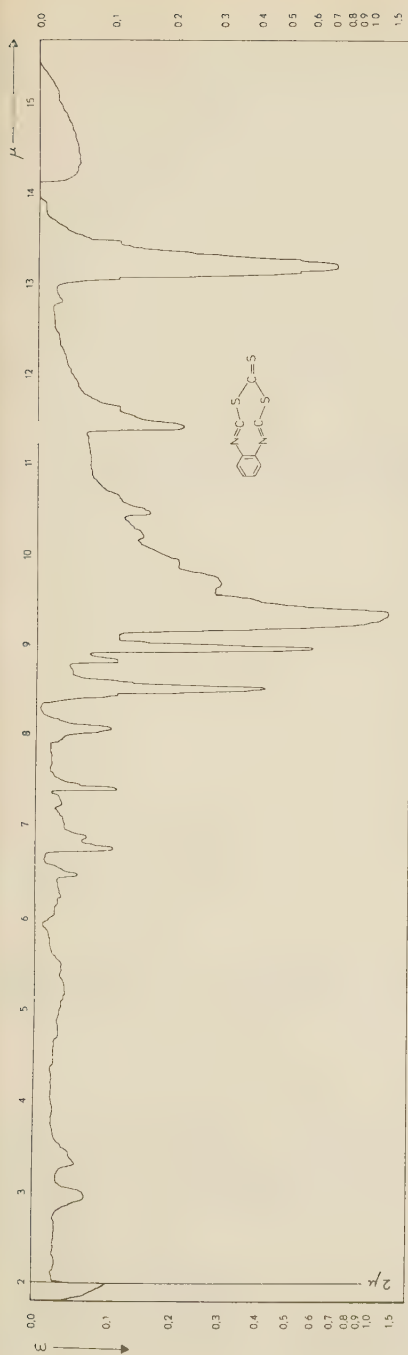
Ss 1451 and Ss 2074 are practically insoluble in water. In most organic solvents, Ss 1451 is not very soluble, whereas Ss 2074 dissolves somewhat more readily.

The striking difference in the colour of the two compounds is also reflected in the considerably varying infra-red spectra⁶⁾. For a more exact comparison,

4) Belg. Pat. 585 312; German Pat. Appl. 27 271 IVa/45 1 (1959), Farbenfabriken Bayer Aktiengesellschaft, Leverkusen, invented by F. Grewe, K. Sasse and R. Wegler.

5) The vapour pressure was determined by Dipl.-Ing. K. Mennicken, Engineering Dept. of Farbenfabriken Bayer Aktiengesellschaft, Leverkusen.

6) The infra-red spectra were scanned by Prof. Dr. M. Pestemer at the Central Research Laboratory of Farbenfabriken Bayer Aktiengesellschaft, Leverkusen.

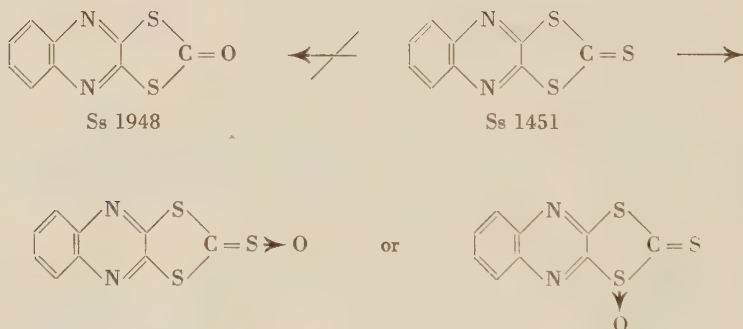


the spectrum of Ss 1451 is shown overleaf together with that of quinoxaline-dithiocarbonate (Ss 1948) which is not methylated in the nucleus.

2. Stability to hydrolysis, oxidation and light

Ss 1451 is a substance with extremely high stability to hydrolysis. The compound is not saponified by 10 % hydrochloric acid and 10 % caustic soda solution even at boiling heat. Cleavage of the trithiocarbonate ring is first effected by alcoholic solution. The dithiocarbonate Ss 2074 is also stable to acids but is quickly hydrolyzed, with ring opening, by aqueous alkalies, especially at high temperature.

The quinoxaline dithiocarbonates (especially Ss 2074) are more stable than the trithiocarbonates (especially Ss 1451) to oxidizing agents. While, for example, Ss 2074 is not changed when reacted with hydrogen peroxide in glacial acetic acid, Ss 1451 readily takes up oxygen under the same conditions and changes into compounds with S-oxide groups, which have biological properties similar to Ss 1451. A change to the quinoxaline dithiocarbonate (Ss 1948) which has less sulphur, was not observed. Nor is this detectable by spectroscopic means for the break-down products that form in field conditions.



Neither Ss 2074 nor Ss 1451 is affected by the influence of light, that is to any detectable extent. When moisture and oxidizing substances are present at the same time, light does, however, tend to increase the speed of break-down ⁷⁾.

3. Toxicity

Ss 1451 has an acute oral LD₅₀ to the rat of 3400 mg/kilo. A daily application of 1/10 of the lethal dosage for a period of 60 days causes perceptible general symptoms but no changes in the blood picture and the urine nor any cases of death are observed. The good skin tolerance of Ss 1451 is evidenced by the fact that no irritation is caused even after 15 to 18 hours of contact with the skin ⁸⁾.

⁷⁾ The stability tests were carried out in conjunction with Dr. H. Kaspers and Prof. Dr. M. Pestemer.

⁸⁾ The toxicological tests were kindly carried out by Prof. Dr. O. R. Klimmer, Pharmacological Institute, Bonn University.

Summary

Ss 1451 (Bayer 30686, ERADEX®) is an absolutely nontoxic, specific acaricide, also very effective against mildew fungi. On account of its mechanism of action which differs from that of hitherto known acaricides, it is highly suitable for controlling spider mites, especially in regions of resistance.

On a new group of acaricides

(Paper presented at XI. International Congress of Entomologists,
Vienna, August 17–25, 1960)

by G. Unterstenhöfer, Leverkusen

A problem which has been intensively investigated during the past decade in all sections of entomology, including classification, is insect resistance to insecticides; we are also pleased to note that more recently close attention has been turned to resistance to acaricides. These efforts are the outcome of valuable findings on the origin, transmission, stability and mechanism of resistance so that our conceptions of the nature of resistance are steadily becoming clearer. The practical object of this research is to establish a basis for systematically devising new methods of controlling pests that have become resistant. When the results hitherto obtained are assessed especially from the aspect of technical research devoted to the development of new products, it must be concluded that no genuine, constructive contributions have yet been made towards solving the problem with chemical products but, on the other hand, valuable information has been furnished for the biological development of new active materials. An interpretation of the findings made by fundamental research, from the technical aspects of application, in conjunction with reliable field observations, reveals which criteria must be especially considered in the biological development and research of new active substances. New acaricides should be expected to possess the following properties:

1. A completely new, primary point of attack in the physiological functions, deviating from that of known active materials, thus making it highly probable that there will be no species-specific differences in reaction and no prospects of predisposition for accelerated development of resistance in regions where resistant populations exist.
2. A high acaricidal power as a result of which reliable effectiveness can be expected so that the intensity of selection, due to relatively rare influence of the acaricide on the populations, will be reduced.
3. A far-reaching selective action in order not to harm beneficial insects and to afford independence regarding the timing of the application, i. e. so that the application can be timed according to the necessities of control technique without having to give consideration, for example, to honeybees.

We systematically set about the task of developing active materials possessing the briefly outlined properties, and in the course of this work we

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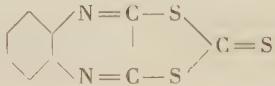
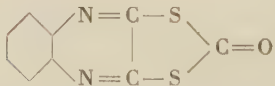
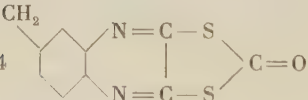
succeeded in discovering a completely new, highly effective acaricidal group, quinoxalines. Derivatives of this group were developed by Sasse and Wegler in the Central Research Laboratory of Farbenfabriken Bayer A. G.

During the very first tests, we found that the group of quinoxalines contained substances with a considerable acaricidal action. For the further biological testing of this active material group, a factor of major importance was that spider mites equally respond to the active compounds, whether of normal, susceptible or resistant strains.

Of the very many compounds that have been prepared and tested, three active materials have so far come to the fore as highly interesting substances. Whereas the development work on Ss 1451, already used in the field under the name of ERADEX®, has largely been concluded, testing of Ss 1948 and Ss 2074 for special purposes will be continued with the aim of utilizing the inherent biological powers of these compounds for phytotherapy.

The following table includes 3 deciding quantitative and qualitative factors for the biological characterization of the compounds in question. The acaricidal power, the factor of prime interest, is expressed, in deviation from usual practice, as that dosage which, under optimum toxicity conditions in the laboratory, eradicates a population consisting of all stages of development. Even though only one numerical value is given to express the acaricidal power of a compound, this indication of effect, which in its character is not so much a toxic value as an expression of efficacy, is nevertheless most accurate and the most reliable criterion also for comparative ratings, in that, as a result, the difficulties arising out of the more or less pronounced stage-specificity in conjunction with the residual action that also influences the toxic value are evaded.

Table 1

Active material	Constitution	Acaricidal power <i>T. telarius</i>		Rat per os	Plant tolerance
		n-mites	r-mites		
Ss 1451 (ERADEX)		0.008%	0.0078%	3400 mg/kg	+
Ss 1948		0.005%	0.0057%	1000 mg/kg	—
Ss 2074		0.0008%	0.00082%	2000 mg/kg	±

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Although a study of the relations between constitution and effect is very productive especially in respect of the group of quinoxalines, this is not possible within the limited scope of this paper. Let it suffice here to point out that as a rule the dithio compounds are biologically more active than the analogous trithio compounds. On the other hand, however, the dithio compounds for the time being rank second to the trithio compounds from the phytotherapeutic aspect because their chemotherapeutic index is less favourable throughout. But as the dithio compounds possess marked ovicidal properties, especially against the winter eggs of the European red mite (*P. pilosus*), which may be of value especially for controlling this pest during vegetation dormancy in regions of resistance, it is still too early to express an opinion on these compounds as pesticides.

Next to nothing is yet known on the mechanism of action in the sense of the primary point of attack during the course of the toxic process. Nevertheless, from observations revealing that the different intoxication phases do not coincide symptomatically with those of the known acaricides, and whose mechanism of action, incidentally, is also completely unknown (1), it may be concluded that the mechanism of action is also specific. Clarification of this would be an especially thankful and perhaps also useful task for biochemical-physiological research insofar as the quinoxalines of interest here are largely selective pesticides which are primarily active against spider mites, and secondarily against fungi, in particular *Erysiphaceae*. Thus, the quinoxalines also strengthen the very highly interesting finding of importance to research — to which March (2) draws attention in his paper — that acaricides often at the same time possess fungicidal properties.

All quinoxalines known to us here are acaricides with an ectotherapeutic action. They lack the translaminar effect or depth action on green plant parts, that is characteristic of most organophosphorus compounds. Hitherto, a systemic action has not been established, either. The corresponding physical prerequisites are wanting for this — the compounds are almost insoluble in water and only slightly soluble in organic solvents.

The quinoxalines are remarkably stable to light and temperature. Therefore, they possess a good residual action, thus guaranteeing reliable effectiveness.

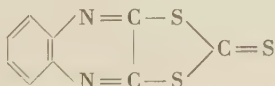
The quinoxaline-2,3-trithiocarbonate referred to earlier under Number Ss 1451 is, as already mentioned, the most interesting active material at present for dealing with resistance to acaricides. Its typically acaricidal properties, such as species-specificity, strain-specificity and stage-specificity including residual action and dependence of the effect on the most important toxicity conditions, as well as the side-effects on plants, mammals and beneficial insects, are sufficiently well-known qualitatively and quantitatively, so that there is nothing to prevent its practical application at first in fruit-growing.

Table 2 provides a summary of the chemism of ERADEX:

Table 2

Active ingredient:	Quinoxaline-2,3-trithiocarbonate
Empirical formula:	$C_8H_4N_2S_3$

Structural formula:



The active substance is a brownish-yellow, loose powder, and practically odourless.

Molecular weight:	236; Melting point: 180° C
Vapour pressure:	1×10^{-7} Torr (mmHg) at 20° C
Solubility	Practically insoluble in water, only slightly soluble in organic solvents.
Stability:	Thermostable up to 200° C, also practically stable in 10 % caustic soda solution and 10 % hydrochloric acid.

An insecticidal action has so far been established only against aphids, with considerable species-specific differences. It has been found to have a perceptible, although weak ovicidal effect against *Ephestia kühniella*, *Bruchidius obtectus*, *Galleria mellonella*.

Important is the unanimous finding of the Austrian Federal Institute for Crop Protection, Vienna and the German Federal Biological Institute (Federal Republic of Germany), Braunschweig that ERADEX is completely harmless to honeybees. In our own tests, we found that *Braconidae* and *Ichneumonidae* are also very resistant.

Its chief pesticidal effect is against mites, especially spider mites (*Tetranychidae*), although *Tarsonemidae* and *Eriophyidae* also react. Just as for all acaricides, there are gradual differences in susceptibility, although not fundamental, among the various spider mite species also in the case of ERADEX. For example, the European red mite (*P. pilosus*), at a concentration level of 0.005 %, is much more susceptible than *T. telarius* which has a threshold value of 0.008 %. The product has been found to have a positive effect also against *T. pacificus*, *M. citri* and *Brevipalpus spec.*

In tests we have carried out to date with the active material on various origins displaying extreme differences in resistance, no strain-specific differences have been found in any species. This strengthens the assumption previously confirmed, that quinoxalines are active materials possessing a new-type mechanism of action.

A special characteristic feature of the acaricide is the more or less marked specific action against certain stages of development. Larvae of *T. telarius* react best to a concentration level (LD_{95}) of 0.006 %, whereas females require a dosage of 0.01 %. The value for eggs lies between these two dosages. ERADEX can, therefore, be termed as a larvicidal-ovicidal acaricide with a marked action against postembryonic stages.

With respect to stage-specificity, the product ranks among those selective acaricides known to have maximum toxicity to larvae (2). Its ovicidal action is primarily unfolded against summer eggs, whereas winter eggs are considerably more resistant and show varying reaction according to age.

After ERADEX has started to act, intoxication proceeds relatively slowly in the postembryonic stages, beginning with arrested movement of the extremities and reduced ability to suck. Larvae and nymphs sometimes may not die until they reach the chrysalis stage especially when the substance is active for only a brief period or when low dosages are applied. The efficacy against post-embryonic stages usually amounts to 80 to 95 % in the first three days after application, not attaining 100 % until during the course of about the next 6

days. The complete eradication of a population is brought about by the residual action against larvae, resulting from the high degree of susceptibility. A striking feature is the plump appearance of dead mites, which persists for a lengthy period.

ERADEX is a stomach and contact poison which, despite its very low vapour pressure, has a considerable effect in the gas phase. It is just in the case of acaricides possessing fungicidal properties that plant tolerance must be very carefully checked. For the product in question, foremost consideration was given to the treatment of orchard fruit, in particular apple, because this fruit sort suffers most in European conditions from attacks by European red mite (*P. pilosus*) which, in turn, reacts very well to ERADEX. This mite, which in certain regions has a high multiple resistance, may be termed as the leading major insect pest of intensive fruit-farming. The product has so far proved to be completely tolerated by 28 tested apple varieties, 12 pear varieties, 2 apricot varieties and 2 cherry varieties. Initial experience also shows that it is tolerated by grape vines, cotton, citrus fruits and carnations, cultures on which resistance to acaricides attaches much importance. It was all the more surprising to find that hops, in themselves very sturdy plants, do not tolerate ERADEX at high relative humidity and during rainy periods, although no plant damage was observed in dry weather.

In extensive field trials carried out in 1959 under very severe infestation conditions brought about by the dry, warm weather, ERADEX gave very good control of both extremely resistant and normally susceptible populations of European red mite in various fruit-growing regions of Europe. An outstanding feature was the unusually long period of freedom from infestation, also in plot tests where the untreated checks and the re-colonized comparison plots exerted great pressure of the threatened infestation on the adjoining ERADEX plots.

Quinoxaline-2,3-trithiocarbonate is thus a selective acaricide equally effective against both resistant and normally susceptible strains of *Tetranychidae*; besides postembryonic stages, especially larvae, it also acts against summer eggs, whereas winter eggs (e. g. of *P. pilosus*) resist it. By virtue of the properties and side-effects that it features, ERADEX is especially suitable for controlling European red mite throughout the whole of the vegetation season including the blossom period.

The other members of the quinoxaline group, quinoxaline-2,3-dithiolcarbonate (Ss 1948) and 6-methyl-quinoxaline-2,3-dithiolcarbonate (Ss 2074), are in all probability valuable phytotherapeutic supplements to quinoxaline-2,3-trithiocarbonate (Ss 1451) insofar as they are also considerably effective, by virtue of their higher ovicidal power, against winter eggs of European red mite and several insect species (e. g. oat-apple aphid), properties which may prove to be of great value for solving the problem of resistance.

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Method of determining residues of systemic insecticides of the METASYSTOX® group in plants

by

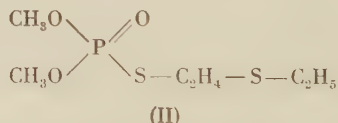
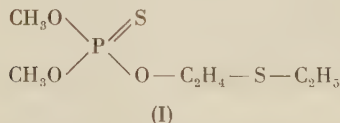
H. Tietz and H. Frehse

Systemic insecticides may be divided into 3 groups, following a suggestion made in 1952 (1):

1. Stable systemic insecticides which are not metabolized by the plant.
2. Endolytic systemic insecticides of which 98 % of the toxic components are present in their original form at the time they are taken up by insects, until the plant finally decomposes them.
3. Endometatotoxic systemic insecticides which are transformed either completely or partially into other toxic substances in the plant, which in turn also have an insecticidal action when they are taken up by insects, until the plant breaks them down into non-toxic substances in the process of metabolism.

METASYSTOX and insecticides chemically related to it (see below) belong to group 3. In order to deal with the question regarding "residues" of such insecticides in plants, it is, therefore, necessary to know the transformation products of the active ingredient concerned that are still insecticidal and thus toxic. The "residues" would then be the sum of all these metabolites.

The original preparation METASYSTOX was a mixture of the 2 isomers (I) and (II), in other words



firstly the "P = S" or thiono form (I), and secondly the "P = O" or thiol form (II) of 2-ethylthioethyl dimethyl thiophosphate, in a mixture proportion of 7:3. Numerous studies that have been carried out with SYSTOX® and also with P = O-METASYSTOX have meanwhile provided a very clear picture of the chemical behaviour of these compounds, both from a purely chemical aspect as well as in biological systems.

In 1951, G. Schrader and co-workers described for the first time the preparation in a pure condition of the oxidation products of the SYSTOX group by direct means*; later, Metcalf and co-workers (2—9), in a series of exhaustive studies conducted at Riverside, California, succeeded in demonstrating that the transformation of the original active materials in biological

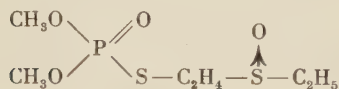
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* cf DBP 876 691 dated 6. 7. 1951
DBP 876 692 dated 7. 7. 1951
DBP 871 448 dated 31. 7. 1951
DBP 947 368 dated 7. 11. 1954

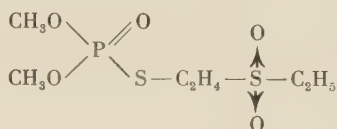
systems is likewise oxidative. After Heath et. al. (10) had discovered transformation products of ISOSYSTOX® in plants, which Mühlmann and Tietz (11) recognized as their sulfoxide (V) and sulphone (VI) using P = O-META-SYSTOX (METAISOSYSTOX®) as an example, it was established, again at Riverside, that in addition 7 transformation products of the 2 parent forms may occur (5):

- (III) the sulfoxide of the thiono form
- (IV) the sulphone of the thiono form
- (V) the sulfoxide of the thiol form
- (VI) the sulphone of the thiol form
- (VII) the phosphate form of the compound
- (VIII) the sulfoxide of the phosphate form
- (IX) the sulphone of the phosphate form.

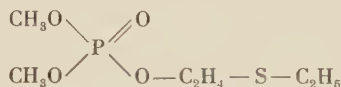
Furthermore, a sulphonium salt has been described by Heath and Vandekar (12).



(V)



(VI)



(VII)

The quintessence of all these studies of metabolism is as follows:

The 2 isomers ("P = S"-form and "P = O"-form) are quickly changed in biological systems into oxidation products some of which are stronger cholinesterase inhibitors and more effective insecticides than the parent substance. These transformation products are chiefly the sulfoxide and, as the result of slower continued oxidation, the sulphone. Starting from the thiono form (I), there are 3 possible ways of this change being brought about:

1. Direct oxidations on the thioether-sulphur atom, which lead to compounds (III) and (IV).
2. Oxidation to the phosphate form (VII) and its continued oxidation to form sulfoxide (VIII) and sulphone (IX).
3. Transformation into the thiol form (II) and its continued oxidation to form sulfoxide (V) and sulphone (VI).

Compound (I), in aqueous solution or heated, now readily changes into compound (II) (13, 14); this process is all the more stimulated in biological systems. P = O-metabolites are formed 5- to 10-times faster by the plant than P = S-metabolites, and are also considerably more stable (4). On the other

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hand, it may be assumed that the phosphate forms (VII—IX) occur in the break-down process only as very short-lived intermediates due to their readiness to hydrolyze. Therefore, compounds (V) and (VI) may be almost exclusively considered as the main metabolites. In the meantime, Heath and Vandekar (12) have traced a sulphonium salt which they have identified as a further transformation product of compound (II), but the part it plays in the biological break-down of the active ingredient has not yet been established. However, due to its great sensitivity to hydrolysis especially in aqueous media, it is always present only in a very small percentage, and so far has been traced only in vitro.

The processes that have been studied in detail with SYSTOX also generally hold good for the other members of this group of substances, as shown e. g. for DISYSTON (15)*, Thimet (15, 16), Thiometon (17), and especially for METAISOSYSTOX (11). The course of break-down broadly outlined above is on principle the same in plants, mammals and insects. This also applies to surface residues which are exposed not so much to biological factors as to physical factors such as sun rays, temperature, moisture etc. It cannot be generally stated in what proportions and when the above-mentioned products of metabolism occur because in this respect external influences and the plant species are factors that have decisive bearing on the outcome (18). The break-down leads to the formation of non-toxic dialkyl-(thio)phosphates and corresponding alcohols.

These findings were in fact partly responsible for the development of the 2 new, improved commercial forms of METASYSTOX: METASYSTOX (i) with compound (II) as its active material, and METASYSTOX (R) which contains compound (V) as its active ingredient to which the product's special advantage of having a considerably less unpleasant smell must be attributed.

All these possible metabolites that have been mentioned have, however, one essential property in common — they can be removed from the aqueous phase by extraction with chloroform (with the exception of the aforementioned sulphonium compound whose occurrence as a transformation product in the process of metabolism is, however, doubtful). The chloroform extract obtained from a harvested plant that has been treated with products of the METASYSTOX group contains, with great approximation, the whole of all those substances which today are referred to as "residues", and whose determination by analysis for the protection of the consumers is just as much in the interests of the manufacturers as it is a matter for legislation.

There are, on principle, several ways of analyzing these residues quantitatively on the required micro-scale, that is after corresponding preliminary purification and separation of interfering attendant substances:

1. Paper-chromatographic separation of metabolites, followed by elution and joint determination; this method could not be evaluated so far because no colorimetric method is known for making a joint determination of the metabolites.
2. A possible way of identifying and also of quantitatively analyzing the residues is to employ the method of infrared analysis: however, very highly purified plant extracts are needed for this sensitive process*.
3. Hydrolytic cleavage of the metabolites followed by determination of the cleavage products — either the phosphoric acids or the alcohols — is a method that offers various possibilities in theory, which, however, could not yet be satisfactorily realised in practice.

* We shall shortly report on experience we have gained on the use of the infrared spectrophotometer for qualitative and quantitative detection of phosphoric ester residues in plant material.

Measurement of cholinesterase inhibition, often employed for lack of specific methods, cannot be considered for practical purposes because of the low inhibitory effect of METASYSTOX metabolites, for in order to obtain a measurable degree of inhibition the plant extracts would have to be concentrated so much that the plant's own substances might effectively inhibit the cholinesterase.

Thus, to have promise of success, it appears advisable to turn to a method which although not constituting a specific analysis for METASYSTOX, does possess the advantage of enabling a quantitative determination to be carried out on micro-scale. This method is based on the principle of determining the phosphorus in the molecule of the metabolites which can be so concentrated without major practical difficulties that the plant-specific phosphorous compounds are largely eliminated. Laws and Webley (19) having already come forward with a method based on this principle, we now present a method built up on experience we have accumulated over many years, and in which we have attempted partly to utilize the method of Laws and Webley.

Our method consists of 2 stages:

- a) Processing of the plant material with purification of the yielded extracts.
- b) Ashing of the concentrated metabolites followed by a microphosphorus determination.

Processing and purification of metabolites

Reagents needed:

Acetone, redistilled

Petroleum ether, boiling range 50—70 ° C, redistilled

Chloroform, redistilled

Magnesium sulphate, A. R., 20 % aqueous solution

Potassium permanganate, A. R., N/20 solution

Sodium sulphate, anhydrous, A. R.

Active carbon, specially prepared: —

Suppliers: Sutcliffe, Speakman and Co., Ltd., Leigh, Lancashire, England.

Specification: Quality No. 207, Type B, Ref. C 5604, Mesh 14—20 BBS.

The carbon is heated to a glow for 2 hours at 600 ° C to 700 ° C in a closed muffle furnace, and then cooled. Afterwards a layer that may have oxidized on the surface is carefully removed. It is now boiled for 1 hour in concentrated hydrochloric acid under reflux, then allowed to cool, and diluted with distilled water. Next, the carbon dust that forms is decanted several times, and the carbon is washed with distilled water until is free of acid. Finally, it is dried for 1 to 2 hours at 110 ° C. Active carbon of other origin, with a grain size of about 1 mm, may also be used provided it is properly prepared, but a preliminary test should be carried out with it to establish whether it is really capable of holding back plant attendant substances and allowing the preparation to pass through. The results given in such a test by different carbons may vary most considerably.

Procedure:

1. Extraction

A sufficient quantity of the harvested crop to be examined should be taken to ensure that good average samples are obtained for the analysis, if possible several kilogrammes, selected from the material in halves or quarters. These samples should be minced in a suitable kitchen appliance, and the

chopped mass carefully mixed. If the substance is green leaf material, place 100 grammes of it in a Waring blender, if fruit take 200 grammes, then add 400 ml of acetone, and macerate for 5 minutes at moderate speed; next, filter the mass through a large suction filter into a 1 litre suction bottle connected to a vacuum system. The dry residue on the filter (less the filter paper) should now be put back into the Waring blender, and again extracted as before with 200 ml of acetone for 5 minutes. Then, using the same suction filter, pour the acetone into the suction bottle, rinse out the blender flask with another 150 ml of acetone, and afterwards use it to wash the residue on the filter, stirring it up gently. After the whole quantity of acetone has been filtered, the residue on the filter will be white or grey and should be discarded.

An unsprayed check sample of the same harvested crop should be treated in the same manner, and correspondingly subjected to the whole process.

The combined acetone extracts of each sample — after having been passed through a fluted filter to remove cellulose residues possibly carried in the mass — should be evaporated under vacuum in a large round-bottomed flask until an aqueous residue is left, using a water bath temperature of between 50 °C and 70 °C. The aqueous residue should not amount to more than 50 to 60 ml, that is when starting with 100 grammes of leaf material, and should be less than 100 ml for 200 grammes of fruit. This avoids any possibility of acetone residues being left in the extract and interfering with the following extractions.

2. Extraction with petroleum ether

The aqueous residue, allowed to cool off well after evaporation, should be poured into a measuring cylinder; rinse out the flask with distilled water, and also pour this into the measuring cylinder until a level of 100 ml is reached. Then extract 4 to 5 times in a separating funnel (size 500 ml) with petroleum ether (100, 50, 50, 50, 50 ml) until the final petroleum ether extract is also colourless. As a rule, 4 extractions will be sufficient. These petroleum ether fractions can be used each time for rinsing out the round-bottomed flask before being poured into the separating funnel. The petroleum ether extracts should be discarded.

Care must be taken, especially in the final extractions with petroleum ether, that no particles or droplets that contain chlorophyll are mechanically carried through when the water phase is drawn off and re-poured into the separating filter, thus escaping the careful extraction with petroleum ether. If necessary, this must be prevented by washing out the equipment with water and acetone; the water that is used here should be added to the water phase already drawn off.

The extraction must be carefully conducted to avoid formation of emulsion. Any emulsion layers that form between the water and petroleum ether should be drained back into the water after the first and second extraction operations; the water must be separated in a perfectly clean state after the third and fourth (also the fifth, if carried out) extractions, and any emulsion residues then present should be discarded with the petroleum ether. If more persistent emulsions occur in the processing of fruits, they can be kept back from the third extraction onwards (if necessary, centrifuge beforehand) by using a fluted filter which should then be washed out several times with a little water. This wash water, too, must be added to the water phase already drained off.

The water extract may have a milky, turbid appearance in the processing of cabbage. This turbidity will not, however, interfere with the remainder of the processing operation provided it contains no emulsion layers.

3. *Extraction with chloroform*

The water phases originating from the extraction with petroleum ether should be combined. Their volume should be determined, and then they should be poured into a clean 500 ml separating funnel, and shaken 5 times with half the volume (based on the amount of water present) of chloroform. The chloroform phases should be combined. If they contain residues of water, the whole extract must be filtered over anhydrous sodium sulphate A. R. These operations with chloroform should be carried out in quick succession. When material with a high content of chlorophyll is used, the chloroform extract will be yellowish to reddish-brown, whereas for fruits it is usually colourless. The aqueous residue is discarded.

The data of Mühlmann and Tietz (l. c., p. 123) disclose that neither the cellulose residue (from stage 1) nor the petroleum ether fraction (from stage 2) nor the water residue extracted with chloroform (from stage 3) contains biological effective components of the product. The chloroform phase contains all metabolites concerned.

4. *Oxidation of pre-purified extract*

Although the carbon column used in stage 5 largely keeps back plant-specific compounds that contain phosphorus, we have registered good success by including an additional oxidation stage especially in the processing of material with a high content of chlorophyll. This oxidation can always be recommended also when the chloroform extract is only slightly coloured. By means of this oxidation stage, the excess pigment, on the one hand, is converted into a form which is retained by the carbon column, and on the other hand, the metabolites present are converted into the respective sulphone form which is more stable, less volatile, analytically more uniform and better to handle.

For this purpose, the chloroform extract is very strongly evaporated by vacuum distillation (as described in stage 1) or also by using a Kuderna-Danish Evaporator, and then the remainder of the chloroform is removed by blowing dry with a light, purified air stream. The residue is then dissolved in 2 ml of acetone; 5 ml of a 20 % magnesium sulphate solution are first added to the acetone solution, then 20 ml of an N/20-potassium permanganate solution, it is now shaken up, and the oxidation solution is allowed to react for 15 minutes at room temperature*. Afterwards it is transferred to a 100 ml separating funnel, and extracted in it 5 times with 20 ml of chloroform each time. The oxidation flask is rinsed out several times in the process with the chloroform that is used. Approximately 2 grammes of anhydrous sodium sulphate are added to the whole chloroform extract; then filter off through a small fluted filter which should be washed out twice with 10 to 15 ml of chloroform. The chloroform extract which had a yellowish to brownish colour before the oxidation, now appears greenish. During the oxidation, it is essential that an excess of permanganate is maintained.

* The method was originally devised by F. A. Gunther and R. C. Blinn (both on the staff of the Citrus Experiment Station of the University of California, Riverside, Calif., U. S. A.) in cooperation with us for the quantitative oxidation of METAISOSYSTOX to the corresponding sulphone for the purpose of infrared analysis. The experiments have not been published.

5. Purification on a carbon column

In this stage, we employed the principle of the carbon column recommended by Hancock and Laws (20) and also used by Laws and Webley (19) for purification.

A chromatographic tube made of glass, which is 18 mm wide and 30 cm long, is provided with a ground-in tap at its bottom outlet; a plug of wadding is firmly inserted up to a level of 1.5 to 2 cm above this tap, and moistened with chloroform. 3.5 grammes of the active carbon are weighed out, placed in a closable, sturdy glass tube (fitted with a suction connection) under chloroform, and aired for 15 minutes under vacuum. It is then inserted together with the chloroform into the chromatographic tube, where the carbon is covered with a small wad.

The chloroform extract obtained in stage 4 should be evaporated to about 5 to 10 ml and placed on the column. The tap of the column should be set for about 2 ml to run off per minute. 100 ml of chloroform should be used altogether for elution, applied at first in small dosages and later in larger ones. The effluent is colourless, any colour that might appear would raise the blank value, and would have to be regarded as faulty. The purification process is now completed, and the extract can be ashed.

Ashing and determination of micro quantities of phosphorus

Reagents needed (A. R.):

Sulphuric acid conc.,	}	for wet-ashing
Nitric acid conc.,		
Perchloric acid 70%,		
Ammonium molybdate solution 2.5 % $[(\text{NH}_4)_6 \text{Mo}_7\text{O}_{24} + 4 \text{H}_2\text{O}]$		
Potassium iodide solution 20 % plus 0.5 % sodium carbonate		
Sodium sulphite 0.5 %		
Potassium dihydrogen phosphate according to Sörensen (Merck) (for plotting the standard curve).		

Ashing

Each processed analytical sample, including the control sample, yields after stage 5 a chloroform extract of somewhat more than 100 ml. These extracts are each divided into 2 exactly equal parts, these halves are then again evaporated almost to dryness, as described above, for a parallel determination, and quantitatively transferred with a little acetone into the glass ashing tubes. Large, thick-walled, heat-resistant test tubes are used as ashing tubes, which are weighed in accurately to ± 1 mg on a not too fine analytical balance. 1 ml of concentrated sulphuric acid and 2 ml of concentrated nitric acid are then added to the analytical sample in the ashing tube, and heated for 30 minutes to 150°C in an electric oven. Next, 0.5 ml of perchloric acid is added, and the excess acid is evaporated with fuming at 300 to 320°C down to 0.5 to 0.6 g. If necessary, the addition of nitric acid and perchloric acid must be repeated. After being allowed to cool down, the sulphuric acid left in the ashing tube is replenished to give 1.25 g (± 5 mg). Rinse with about 15 ml of distilled water into a 25 ml measuring flask, add 2.5 ml of the molybdate solution and

* This is an adapted method of determining micro quantities of phosphorus, which has been used in practice for many years in the described form by the Organic-Analytical Laboratory of Farbenfabriken Bayer AG.

2.5 ml of the iodide solution, and heat for 15 minutes in a boiling water bath. A green mixed colour of molybdenum blue and iodine is formed.

The flask is allowed to cool, and the liberated iodine is reduced with sodium sulphite, i. e. until the colour changes to blue, and then an excess of 0.5 ml sulphite solution is added. Fill up to the mark with distilled water, and measure in a 5 cm cell at 750 m μ (Zeiss Elko II) using a blank comparison solution prepared from a mixture of the above quantities of reagents treated in the manner described above and diluted to 25 ml. The absorption maximum of the phosphomolybdenum blue colour, however, is at 840 m μ . The method enables between 0.02 and 0.4 γ /ml phosphorus to be determined with the aid of a suitable standard curve which is best plotted with an inorganic, analytically pure phosphate with inclusion of the ashing process.

We have found from experience that in certain circumstances the temperature and duration of ashing have an influence on the recovery, which may result in a loss of up to 30 % maximum. In order to keep such possible losses under control, a blank experiment with an oxidized preparation should be made before carrying out the process described in this paper.

Blank values and recoveries

The phosphorus blank values which are obtained with the method described here from plants that are not treated with phosphate insecticides, determine the sensitivity of the method.

Our experience has shown that plant-specific extractive substances which survive the described process of purification display seasonal variations in their quantity in the sense that "winter leaves" tend to give higher blank values than "summer leaves" or "autumn leaves".

We have devised our method mainly on 3 models: white or savoy cabbage, beet leaves and apples.

On expressing the P-blank value as METAISOSYSTOX-sulphoxide (factor = $P \times 7.95$), we obtained the following blank values, as the means for numerous processings:

Cabbage: 0.20 ppm METAISOSYSTOX-sulphoxide

Beets: 0.18 ppm METAISOSYSTOX-sulphoxide

Apples: 0.02 ppm METAISOSYSTOX-sulphoxide

For additions of 1 or 0.5 ppm of sulphoxide to the plant macerate in the Waring blender (start of stage 1), our average recoveries were as follows:

86 % for cabbage

70 % for beets

84 % for apples.

As the artificial addition of active material to a plant macerate cannot be compared with a natural active ingredient depot following a spray treatment, we carried out experiments in early summer this year to control the method described here in practical application, using a P³²-labelled METAISOSYSTOX preparation. The active ingredient was formulated to correspond with the 25 % METASYSTOX (i) product available on the market, and was applied in 2 series of tests.

On June 1, sugar beets were sprayed at a rate of 800 ccs/600 litres/hectare (approximately 12 fl. oz./50 gal./acre) and lettuce at a rate of 600 ccs/600 litres/hectare (approximately 9 fl. oz./50 gal./acre). Samples were taken in the field 1, 3 and 6 days after the spray treatment, and immediately analyzed.

These experiments were merely designed as a methodical test so that they were not extended beyond the 6th day after spraying. The following table gives the traced residues in ppm and the recoveries obtained. The recovery is understood to mean the results of the P^{32} -count following the complete processing operation, based on the activity of the chloroform-soluble metabolites. It is thus shown that no appreciable losses are caused by stages 4, 5 and 6 of the procedure.

Day	Lettuce		Sugar beets*	
	ppm	% recovery	ppm	% recovery
1st	4.67	85.0	8.58	96.7
3rd	3.35	98.0	6.36	100.0
6th	0.75	105.0	0.91	87.5

* Leaf analysis.

Summary

A method is described by means of which residues of insecticides belonging to the METASYSTOX group can be determined to values of below 0.5 ppm. The harvested crop to be analyzed is subjected to a preliminary purification by extraction with organic solvents, the extracts are purified on a carbon column, wet-ashed and analyzed for their content of phosphorus in micro quantities.

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Addendum

We have meanwhile established that an additional purification stage may be necessary for some special crops such as hops and potato tubers.

Hops. Commercial hop cones are cleaned-up until a chloroform extract is obtained (incl. Stage 3). The chloroform extract is evaporated to dryness, and then redissolved in 500 ml of acetone to which 0.5 ml of a saturated methanolic calcium chloride solution is added. The flask is placed in a mixture of ice and common salt for 1 to 2 hours to precipitate phosphatides. Then filter through a fluted filter with filter aid, and rinse the flask and the filter with a little acetone. The residue on the filter is discarded, and the acetone distilled off in vacuum. The residue from the acetone phase is purified on acid aluminium oxide (for chromatography, Merck) as follows: Make a chloroform slurry using 35 grammes of aluminium oxide and pour this into a chromatographic tube of the type described in Stage 5. After the adsorbent has settled, a column approx. 20 cm high is obtained. The dry residue in the flask (see above) is taken up with as little chloroform as possible (but no more than 10 ml) and applied to this column. After the chloroform has penetrated into the column, another 100 ml of chloroform is passed through.

The chloroform that emerges from the column is then treated in the usual manner, beginning with Stage 4. The recovery is not affected by these additional stages.

Potatoes. Following oxidation with permanganate (Stage 4), the chloroform phase often becomes brown; this pigment is not retained by the carbon column. In order to eliminate this deficiency, it is recommended, before the oxidation stage, to purify the residue on aluminium oxide, as described for hops. The recovery is not affected by this additional stage.

Histological Studies on Vetch Aphis (*Megoura viciae* Buckt.) after Application of SYSTOX®

by

Günther Voss and Peter Ehrhardt

Introduction

Histological methods have been applied by numerous authors in studies conducted to establish on which part of the insect organism an insecticide acts. Hopp (1953) investigated the action of several breathing and contact poisons of the chlorinated hydrocarbon group on the organs of the body louse. Gösswald (1958) discovered histological changes in the thoracic ganglion of the yellow-necked termite *Calotermis flavicollis* Fabr., and Klee (1959) on the intestinal epithelium of the same species after application of Thiodan. The influence of E 605® on different organs of treated insects has been examined by Lüers, Köpf and Lüers (1953), Pistor (1954, 1958) and Jochum (1956), whereby in the first of these papers reference is merely made to changes in the nervous system (thoracic ganglia and sense organs). Jochum, on the other hand, is of the opinion that increased glandular activity in the digestive tract of treated insects attaches great importance. Wiesmann (1957), in his comparative studies on normally susceptible strains and DDT-resistant strains of house flies, discovered striking differences in the epithelial cells of the tarsal hypodermis and in the basal cells of abdominal hairs. This perhaps best illustrates the bearing which the state of a cell has on uptake of poison and the poisoning process. In view of this, exhaustive histological studies are fully justified.

Formulation of problem

The authors mentioned in the introductory paragraph examined the insecticides they used, as contact and breathing poisons; in each case, the poison was applied externally. By way of contrast, we shall report on the action of a systemic insecticide (SYSTOX®), for the use of such a product guarantees satisfactory peroral uptake in the intestinal tract of plant-sucking insects. With this method, the place of entry for the poison and the site of initial action are exactly localized, whereas the conditions for application of contact and breathing poisons frequently vary; such conditions are not always comparable, and often make a satisfactory interpretation difficult. Furthermore, aphids take up the strongly diluted insecticide from the plant, together with their food, under natural conditions.

In a previous paper (Ehrhardt and Voss, 1960), the gaseous metabolism was studied in *Megoura viciae* Buckt. (*Aphidoidae*) under the influence of E 605 and SYSTOX. Differences were observed in the uptake of oxygen following external and internal (peroral) application of SYSTOX. The obvious reaction

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would be to attribute this phenomenon to the insecticide being active at different sites; exact histological studies appear to us as constituting a suitable method for clarifying these questions. However, on drawing a comparison with the results of Jochum, it is most essential to give consideration to the special physiological relations of the aphids, to which reference was already made in the first paper.

Material and methods

The studies were conducted on apterous imagines of *Megoura viciae* (Buckt.), bred in the laboratory, and kept under constant conditions (long-day lighting; temperature of 20—22° C) on *Vicia faba*. The peroral application was carried out by allowing the aphids to feed on *Vicia faba* plants that had stood overnight in a 0.1 % solution of SYSTOX. A macroscopic examination showed the product to have no phytotoxic effect for the duration of the experiment. The insects dropped from the plants about 3 hours after starting to feed, and a little time later the aphids were in an irreversible knock-down position (lying on their backs). One batch of the aphids was at once fixed in this position, and another 20 hours later. As a comparison, feeding aphids were treated with 20 mg of evaporating SYSTOX in a closed vessel — the possibility of direct contact poisoning was precluded — and then likewise fixed in a knock-down position lying on their backs. Untreated check aphids were taken from fresh plants at corresponding intervals, and also fixed.

Histological technique: Our procedure for fixing the aphids was as follows. The aphids were pre-fixed under vacuum in Carl's mixture heated to 60° C, and were then placed in fresh fixative for 24 hours at room temperature and under normal pressure. Imbedding in paraffin was carried out by the usual method. The 5 μ thick sections were stained with Haemalum-Eosin, hematoxylin (after Heidenhain) and by the Azan Stain method (Romeis). The series of sections obtained from the differently treated aphids and the untreated checks were mounted on one and the same slide so that they would be left in the staining and differentiating solutions for an equally long period. A modified carmine-acetic acid method was used for determining the nuclear sizes. The photographic exposures were made with a Zeiss plate attachment camera.

Histology of digestive organs of untreated aphids

In order that histopathological changes, brought about by a SYSTOX application, can be distinguished more clearly from the normal picture or condition, some details will be furnished here on the structure and histology of the examined organs in healthy aphids. Besides our own observations, consideration has especially been given to the data contained in the paper of H. Weber (1928) on the bean aphid (*Aphis fabae* Scop.).

The digestive tract of *Megoura viciae* is divided as follows: — The oesophagus, an evenly thin tube, opens into the gizzard at the *Valvula cardiaca*. According to Weber, the mid-intestine consists of 2 sections with strictly separate functions, the gizzard and the very long small intestine. Also in the starved individual, the gizzard is a tightly filled, oval sac which is slightly constricted in the centre. This organ is alone responsible for the secretory action in the digestive process, being clearly recognizable in the histological

picture from segmented secretion balls. The phenomena of secretion, however, never appear simultaneously in the whole of the epithelium of the stomach, but occur at regular intervals in the form of waves spreading from the front to the rear end. In the stage of rest, the stomach cells are usually flat and hexagonal; the likewise flat nucleus causes the cell to curve forward slightly. After application of the azan stain method, the nuclei appear structurally as finely granulated violet bodies, and the nucleoli are bright red, whereas the cell walls assume a faint blue colour. Following this stage of rest, the cells enter into different phases of apocrine secretion. The gizzard passes almost unnoticeably into the long small intestine that consists of 4 loops. The wall of this organ comprises 5 to 7 adjoining cells which in cross-section appear to have a blunt, cone shape. Their tips protrude far into the interior of the intestinal lumen so that this is restricted to very narrow gaps. As a result, the enlarged surface is produced that is necessary for a resorptive organ. The cytoplasm has the appearance of a wide-meshed, granulated net which is tighter at the cell boundaries, and considerably slack in the central part of the cell around the nucleus. In contrast to the resting cell nuclei of the gizzard epithelium, the nuclei of the small-intestinal epithelium have a round shape (Fig. 1). Due to the wanting secretory functions, they present the same picture throughout the small intestine. The hind-intestine (rectum) joins the small intestine as a very thin-skinned tube that is capable of expanding, and which has longitudinal folds when empty. Its flat cells are not clearly marked off from each other; the nuclei are flat and possess nucleoli which become clearly prominent when stained.

Change in the histological picture after uptake of SYSTOX

No changes were observed in the cells of the oesophagous wall. An interpretation of the histological changes in the gizzard epithelium, perceptible in several cases, meets with difficulties for the following reasons: The nuclei are

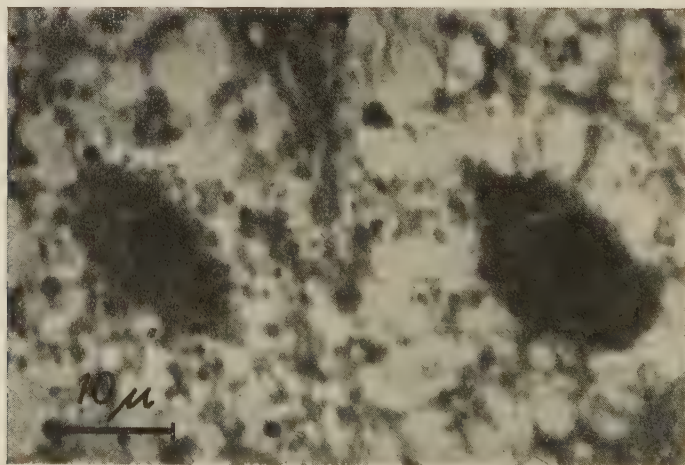


Fig. 1: Cells taken from the small-intestinal epithelium of an untreated *Megoura viciae* (Buckt.) individual, shown in longitudinal section. Cell nuclei, nucleoli and the net-like structure of the cytoplasm are clearly seen

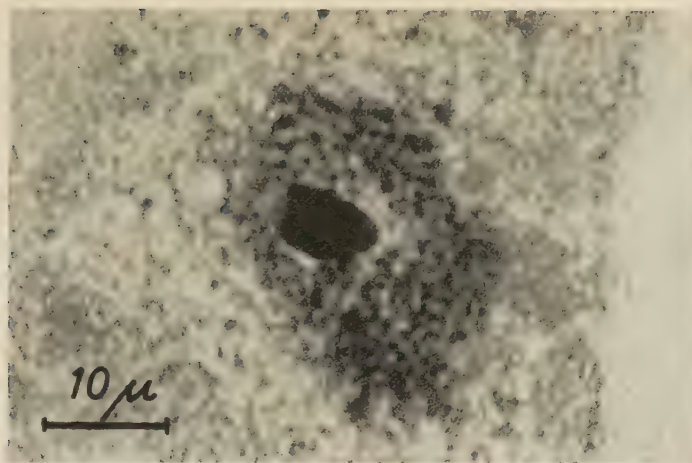


Fig. 2: 1st degeneration stage of small-intestinal cells of *Megoura viciae* (Buckt.) following peroral uptake of SYSTOX. Cell nucleus swells, nucleolus remains unchanged, net-like structure of cytoplasm disintegrates into a homogeneous state

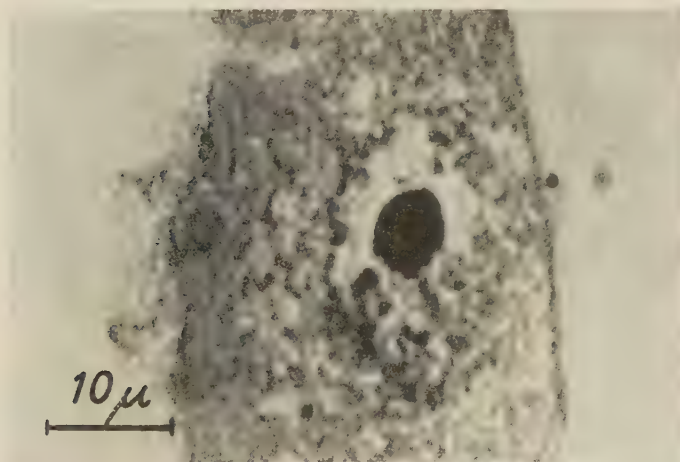


Fig. 3: 2nd degeneration stage: — disintegration of cell nucleus (karyorrhexis) and considerable loss of staining ability (chromatolysis). The nucleolus remains unchanged

subjected, as mentioned in the preceding section, to a constant change due to their secretory function; this change takes the form of a modification in shape and varying affinity to the histological stains. As the gizzard cells have no resorptive action whatsoever, it is assumed that poisoning symptoms occur

here only in a reduced form, and that the gizzard has no particular influence on the ultimate outcome of the primary poisoning process.

In contrast, far-reaching changes were established in the small intestine following a peroral uptake of SYSTOX. The first clearly perceptible abnormality is initiated by a disintegration of the net-like structure of the plasm (Fig. 1) into a largely homogeneous state, in which the whole of the cell lumen is evenly filled with cytoplasm (Fig. 2). At the same time, a blurring of the cell boundaries is noticed. During this process, the first changes in the cell nucleus are already observed; the nucleus enlarges most perceptibly, while the size, shape and colour of the nucleolus remain completely constant. The nucleus may swell to 2—3 times its normal volume. This expansion is accompanied by a disintegration of the nucleoplasm and of the chromatin. Finally, the nucleus is completely disintegrated (Fig. 3). The nucleolus is at first preserved although in

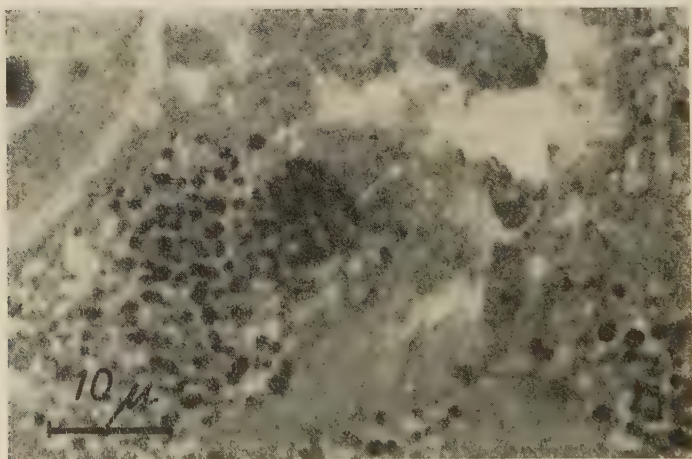


Fig. 4: 3rd degeneration stage. Swelling and disintegration of nucleolus

this stage it frequently appears somewhat enlarged. Fig. 4 illustrates the subsequent disintegration of the nucleolus which ultimately terminates in complete degeneration. In this phase, following azan staining, the red nucleolus substance is diffused throughout the whole of the cell lumen, this being especially noticeable in Fig. 5. The degenerative state of the cell and its inclusions is thus obtained.

The different stages of nuclear disintegration are spread over the entire length of the small intestine; the final stages are encountered almost exclusively in the anterior section. Disintegration is less pronounced towards the hind-intestine where the nucleolus usually remains unchanged. The nuclei of the hind-intestine itself are merely swollen, with no changes in the nuclear structure and the nucleolus.

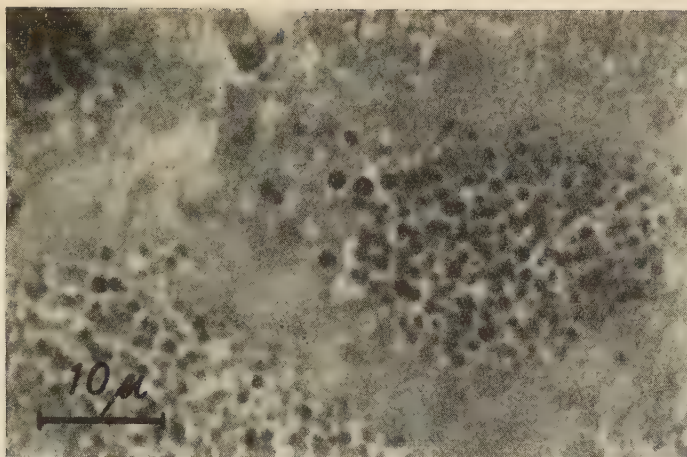


Fig. 5: 4th degeneration stage (final stage in a small-intestinal cell of *Megoura viciae* [Buckt.]) poisoned with SYSTOX. Nuclear substance and nucleolus completely disintegrated; components of nucleolus still visible as dark, round granules. Karyolytic stage

In order to determine the above-mentioned change in the size of the nuclei and nucleoli of the small-intestinal cells more accurately, measurements were carried out. The intestinal tract was dissected out in Ringer's solution, fixed for 12 hours in alcohol-glacial acetic acid (3:1), and stained for 12 hours in carmine-acetic acid. This was followed by brief differentiation in 50 % acetic acid, and then crush preparations were prepared on slides in a mixture of lactic acid, Orcein (Messrs. Merck) and glacial acetic acid. The measurements of size are based on measurements in oil immersion and phase contrast observations.

Table 1: Changes in size of cell nuclei and nucleoli in small-intestinal epithelium of *Megoura viciae* following peroral uptake of SYSTOX. The figures given are means calculated for 100 individual measurements.

	Control	3 hrs. after uptake	14 hrs. after uptake	25 hrs. after uptake
Nucleus (lengthwise)	37.9 my	40.5 my	47.3 my	(28.4 my)
Nucleus (crosswise)	21.9 my	29.4 my	33.0 my	(21.1 my)
Nucleolus (diameter)	9.7 my	21.2 my	16.8 my	(11.8 my)

Just as already revealed by the described histological findings, the values given in Table 1 also provide evidence of initial enlargement of the nucleus and nucleolus (swelling). The subsequent degeneration becomes evident, also with

this method, from a marked diminishing of the nucleus size and a disintegration of the chromatin. The values given in brackets are only approximately correct for in an advanced stage of poisoning the boundaries between the nucleus and the cytoplasm become blurred, often making exact measuring impossible.

Initial nuclear degenerations were observed only in the small intestine of aphids fixed immediately after they had fallen from the poisonous plant.

None of the above-mentioned symptoms appears in the intestinal cells after application of SYSTOX as a breathing poison. The plasm and nucleus do not differ from those of the untreated check.

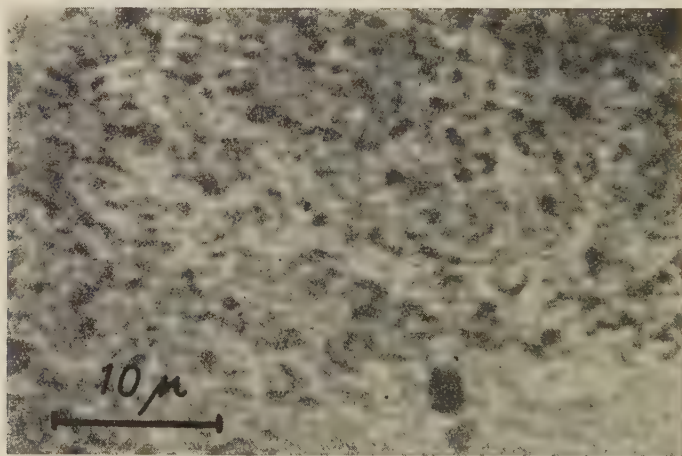


Fig. 6: Longitudinal section through the peripheral zone of the cerebral ganglion of an untreated *Megoura viciae* (Buckt.) individual. The nuclei of the nerve-cells are indistinct

Following peroral uptake of insecticide, no abnormality was observed in the peripheral cell nuclei of the cerebral ganglion. In the normal picture, the following structures are recognized following azan staining: The nuclei of the neurones stand out but little from the surrounding plasm (Fig. 6). The cells are extremely small, and are situated very close to each other. Due to the smallness of the objects, further details could not be recognized even with greatest magnification by means of oil immersion. However, after application of SYSTOX by inhalation, the neurones shrink most considerably so that gaps are perceptible between them (Fig. 7), and the nuclei appear to fill almost the whole of the cell lumen, this being clearly reflected in the increased staining ability. The conglomeration of the chromatin may be interpreted as pycnosis.

The greatly reduced fat-body of the aphids consists, in a normal condition, of coarsely vacuolated cells with very stainable, compact nuclei which, following application of breathing poison, become larger and disintegrate. The vacuoles in the cytoplasm become smaller as the intoxication phase progresses, and then completely disappear until finally the plasm evenly fills the cell lumen.

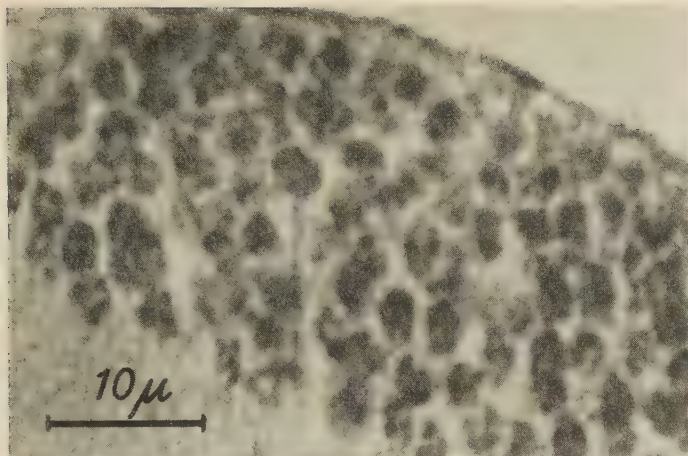


Fig. 7: Nerve-cells in cerebral ganglion of *Megoura viciae* (Buckt.) following application of SYSTOX as a breathing poison. Shrinkage of cells, conglomeration of chromatin, and increased stainability of nucleus (pycnosis).

Contraction is evident in the muscular fibres following both peroral uptake of poison and application of poison by inhalation (Figs. 8 and 9). The alternating isotropic (incl. Krause's membrane) and anisotropic portions in the cross-striated muscle show a shortening of the isotropic layers in a contracted state,

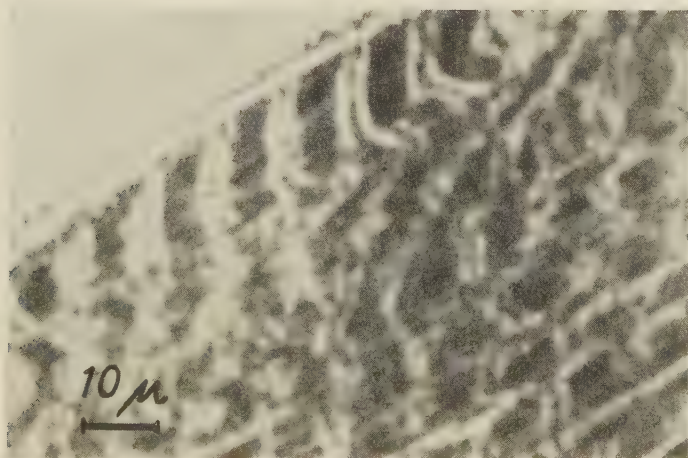


Fig. 8: Leg muscle of 1st thoracic segment in an untreated *Megoura viciae* (Buckt.) aphid. The light isotropic portions, together with Krause's membrane, are not contracted.

whereas the anisotropic zones remain unchanged. The contraction of the individual muscle fibrillae, caused by the influence of poison, is usually not homogeneous; rathermore, the adjoining intermediate membranes are no longer on the same level, thus creating the impression of a zigzag line.

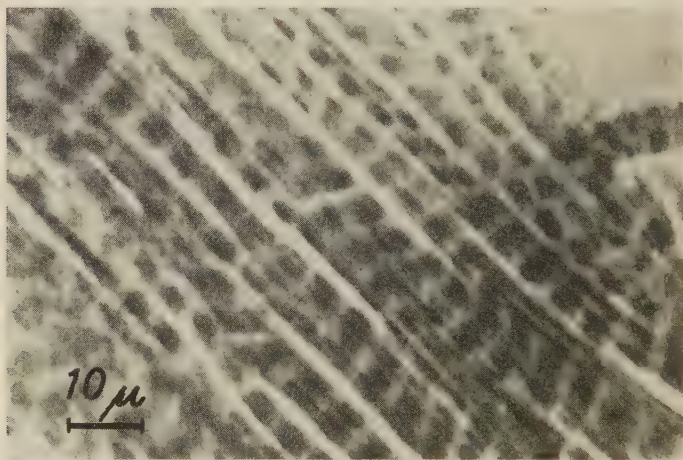


Fig. 9: Leg muscle of 1st thoracic segment in a *Megoura viciae* (Buckt.) aphid treated with SYSTOX. The isotropic portions appear greatly shortened due to contraction

No changes were observed in the salivary glands, either in the plasm or in the nuclei. The numerous larvae in different stages of development present in the abdomen are not harmed, either, by treatment with SYSTOX at the concentration quoted by us; in our opinion, the poison is kept away from them by the protective integument that surrounds them.

Discussion of results

Before discussing the results of our studies and comparing them with findings of other authors, we shall firstly consider generally the possibilities and limitations of histological examinations with respect to their usage for clarifying questions relating to the mechanism of action of an insecticide and to the site at which it acts in the organism. The histological working method employed in our studies does not permit statements to be made on the primary changes of poisoned, degenerating cells. We are of the opinion that the symptoms of cell degeneration apparent in the histological picture are due to a physiochemical change in the state of the cell substances brought about by

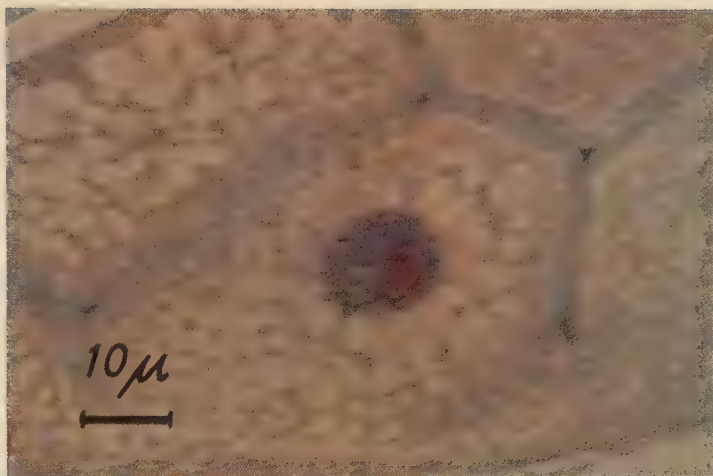


Fig. 10: Colour reproduction of an untreated small-intestinal cell of *Megoura viciae* (Buckt.), corresponding to Fig. 1.
(Agfacolor Negative Cut Film CT 17, 20 sec. exposure)

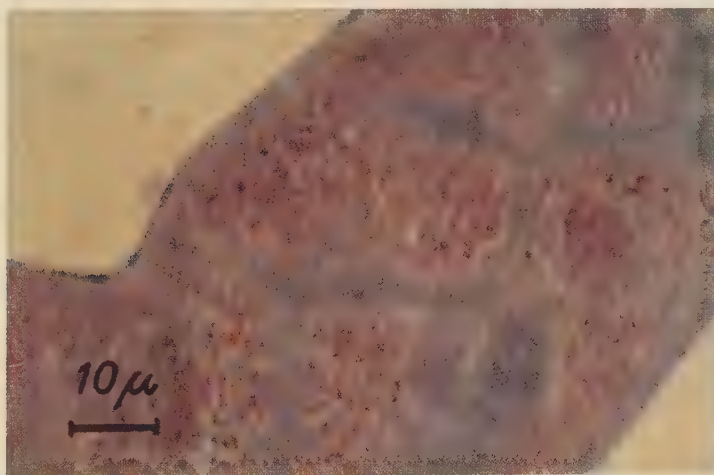


Fig. 11: Colour reproduction of SYSTOX-treated small-intestinal cells of *Megoura viciae* (Buckt.) Karolytic stage, corresponding to Fig. 5.
(Agfacolor Negative Cut Film CT 17, 20 sec. exposure)

the insecticide, which in turn leads secondarily to the histologically fixable chain of irreversible symptoms of degeneration. Apart from numerous other methods, the histological method, therefore, also fails to qualify as a working

method for studying the chemical mode of action of an insecticide in the organism. This method, however, does achieve excellent results in cases where it is a matter of establishing the site of action and the gradual translocation of a preparation in the insect organism, provided of course that the action of the poison is at all manifested by a change of the histological structure, which by no means need always be the case.

In view of this possibility, it could definitely be established that the points at which SYSTOX acts in the aphid organism, following external and peroral application, are localized at different sites; we had already assumed this to be the case following studies of the gas metabolism in treated aphids.

When aphids take up SYSTOX from the plant, the following changes may be established in the small-intestinal epithelium: the plasma changes from a coarsely vacuolated state into a homogeneous state, and is now evenly distributed throughout the cell; the cell walls become indistinct and blur. At the same time, the normal cell nucleus is considerably enlarged (swelling), the chromatin stains less readily (chromophobia), this being ultimately followed by the stages of nuclear disintegration (karyorrhexis), chromatolysis, swelling of the nucleolus, disintegration of the nucleus and the eventual complete disintegration of the nuclear substances (karyolysis). In this phase, the chromatin completely loses its stainability. Once the cell, together with its inclusions, has reached the final stage of degeneration, it has already become incapable of functioning, i.e. it is no longer able to resorb substances from the intestine. In all other organs, however, no pathological changes were observed even after 24 hours of knock-down (aphids lying on their backs). Conditions of contraction of a varying degree were observed merely in the cross-striated skeletal muscles in practically all cases (shortening of the isotropic portions). This symptom is certainly associated with the disturbing effect of the insecticide on the transmission of excitation in the nerve-muscular system, the final stage of which, following excessive excitation, is paralysis. On the basis of these observations, we explain the process of poisoning as follows:

After the poison has been taken up in the intestinal tract, it is resorbed by the small-intestinal cells and passed to the hemolymph which transports the poison to other sense organs. The uptake and distribution of the insecticide in the insect body must proceed at great speed for after feeding for only 3 hours the aphids drop from the plants due to the described symptoms of paralysis. The passage of SYSTOX to the hemolymph can of course only continue as long as the cells of the small-intestinal epithelium are still intact, i.e. are able to resorb the intestinal contents. We attribute the final cause of death firstly to the inability of the degenerating small-intestinal cells to function, and secondly to immobility thus rendering it impossible for the aphids to take up food from the plant. In other words, the aphids starve. This conclusion is further strengthened by the observation that following peroral uptake of poison all the treated aphids still showed slight, tremulous motions of their extremities and antennae even after 48 hours of lying on their backs. These movements did not finally cease until after about 55 to 60 hours. Untreated control aphids, too, are only capable of surviving for about the same length of time without food. Reference to this interesting phenomenon was already made in the first paper mentioned earlier.

Apart from these symptoms regarded by us to be the main cause of death, we frequently observed, especially in the second stage of knock-down (24 to

48 hours), a discharge of honeydew from the anus and a secretion of fluid from the genital orifice in poisoned individuals. Jochum regarded this to be the cause of death for the objects he examined, but in our case it appears to be only an accompanying symptom for the histological changes described by us cannot be interpreted as increased glandular activity in the sense put forward by Jochum but rathermore as a far-reaching degeneration and resultant inability to function of the small intestine which is responsible for the resorption of food and the regulation of the water balance (lack of Malpighian tubes).

In complete contrast to this, the symptoms of pycnosis observed in the cerebral ganglion of aphids treated with SYSTOX applied as a breathing poison, point to a central site of action in the organism. The insecticide effective in the vapour phase can act to an increased degree on the central nerve-system through the numerous tracheae and tracheoles. But here, too, the symptoms of paralysis and the resultant inability of the insects to take up food, in our opinion constitute the cause of death.

Summary

1. The structure and the histology of the intestinal tract of *Megoura viciae* (Buckt.) are described, including details on the peculiarities of the intestinal tract in this aphid species.
2. Following peroral uptake of SYSTOX from the plant, progressive nuclear degenerations are observed in the cells of the small-intestinal epithelium in poisoned aphids. The final stage is characterized by complete disintegration of the nuclear substance and the nucleolus. These symptoms are interpreted as karyolysis. No changes are observed in the central nervous system (cerebral ganglion).
3. Following application of SYSTOX as a breathing poison, pycnotic symptoms appear in the cell nuclei in the peripheral zone of the cerebral ganglion. The histological picture of the small-intestinal epithelium does not differ from that of untreated aphids.
4. A contraction of the cross-striated muscles, especially the leg muscles, is observed following both methods of application, being recognized by a shortening of the isotropic portions.
5. The results are finally discussed. The opinion is maintained that degeneration of the small-intestinal cells which are responsible for resorption of food and water balance, is the main cause of death in aphids poisoned by peroral uptake of insecticide. Besides this, however, it is believed that immobility, a condition brought about by the action of the insecticidal preparation, and the inability of the insect to take up any more food, are factors of bearing.

We would express our extreme gratitude to Prof. Dr. K. Gösswald and to Dr. W. Kluft (University Lecturer) for the constant interest they displayed in our work, and for the kind assistance they rendered us.

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Use of Helicopters for Pest Control

by Hasso von Eickstedt

Compared with the large number of fixed-wing aircraft used for protecting intensively cultivated crops, very few helicopters are employed in agriculture. One of the main reasons for this is that after the last war, numerous trainer aeroplanes were available for purchase at low prices, and were modified by many firms for applying insecticides and herbicides by installing dusting and spraying equipment in them.

In agricultural regions where aeroplanes can be used for treating expansive plantations without losing much time on turning manoeuvres, and where runways are available for take-off and landing, the fixed-wing aircraft is still today a very popular machine for applying pesticides especially as relatively large acreages can be treated in a short time and at reasonable costs.

On the other hand, in crop-growing regions where there are no take-off and landing facilities for small aeroplanes, helicopters can often be used especially when it is possible for tank trucks carrying supplies of spray solution and fuel to be driven close up to the plantations.

The majority of helicopters used in the past for crop protection work were equipped with petrol engines — usually fitted behind the pilot's seat — for driving the rotor.

The "Kolibrië" type helicopter (of NHJ, Rotterdam) illustrated in the following photos, is driven by 2 ram jets attached to the ends of the rotor; by combustion of kerosene or similar low-priced fuels, a powerful propulsion is generated in these jets causing the rotor of 10 metre diameter to be revolved at high speed (approximately 400 r. p. m.).

The consumption of fuel which is considerably cheaper than petrol, is relatively high in relation to the mechanically utilized energy, and amounts to about 120 gallons per flying hour.

The extent to which a helicopter fitted with ram jets can compete from the cost aspect with conventional type fixed-wing aircraft (e. g. Piper Cub or Stearman), largely depends of course upon the price of the fuel — needed in large quantities — on delivery at the site of operation.

The purchase price of a helicopter fitted with ram jets is, however, lower than that of a conventional type helicopter driven by petrol engine.

As shown in the photos, the helicopter is equipped with four 100 litre capacity tanks, 2 for fuel, and the other 2 for spray solution (200 litres).

This volume of spray solution, forced-fed into the nozzle boom by means of a small, separate petrol motor, is sufficient to spray approximately 5 to 6 hectares (12 to 15 acres) of field crops per flight. As the spray boom alone has a width of 53 ft. (16.15 metres), this helicopter attains a swath of 62 to 65 ft. (18.90 — 19.80 metres) in flight. Spray droplets entering the zone of the powerful downblast are forced deep into the foliage of the treated plants. Due to the low forward speed of the helicopter, which can be varied between 15 and 50 m. p. h. (25 to 80 kilometres per hour) as required, the average droplet diameter is larger than that sprayed from the faster flying fixed-wing aircraft; the spray



Fig. 1: "Kolibrie" type helicopter after landing. Ram jets are clearly recognised at the ends of the rotor.



Fig. 2: Filling the tanks of a helicopter at the edge of a rice field. The tank truck seen on the left carries both fuel and spray solution, pumped into the tanks of the helicopter through 2 separate pipes. The rotor remains in motion during the tanking operation



Fig. 3: Spraying the rice field. The photo clearly illustrates the 4 tanks for fuel and spray solution, and the nozzle boom



Fig. 4: Helicopter turning. In contrast to fixed-wing aircraft, a helicopter can turn through 180° at the ends of the field in 8 to 10 seconds. Above the tanks (left-hand side) is a small petrol motor for driving the pump which feeds the spray solution into the nozzle boom



Fig. 5: When the spray solution tanks are empty, the helicopter lands directly alongside the tank truck to re-fill with spray solution and fuel; while the helicopter is at work, the tank truck slowly advances up the road

droplets emerging from the nozzles of the latter are additionally broken down by the strong slip stream.

The photos shown here were taken during the treatment of a rice field in Central Cuba; in this operation, METASYSTOX (i)® was successfully used for controlling the leafhopper species "Sogata", a carrier of the virus disease "Hoja blanca".

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Control of Pine Sawfly (*Diprion pini*) in Forests

by Herbert Goeldner, Munich

1. Experimental area, weather, time of spraying:

During the course of a large-scale control operation against pine sawfly, tests were carried out in Central Franconia with DIPTEREX® (50 % active substance) applied at different volumes. The experimental areas included three plots of 30 to 35 year old pines (10 to 15 metres high), interspersed with young trees (1 to 3 metres high). The experimental areas were so selected that they could be compared with each other in respect of age of the stand, stand density and height, distribution of young trees within the experimental areas and pine sawfly infestation.

The experimental areas were treated as follows:

Area I (5 hectares)	treated with 1.5 litres DIPTEREX/hectare (50 % active substance) in 40 litres of water/hectare.
Area II (3.5 hectares)	treated with 1.5 litres DIPTEREX/ha (50 % active substance) in 15 litres of water/ha.
Area III (5 hectares)	treated with a comparison product.

The tests were carried out on July 16, 1960 between 7.30 and 8.15 a. m.

Weather at the time of the treatment: slight wind (2—3 metres/sec.), fairly high relative humidity of over 70 %, sky completely overcast, temperature of 18° C.

The weather conditions were thus favourable for a control.

Following the spray application, the clouds broke up; towards mid-day the weather was cloudy and bright, in the afternoon bright and cloudy, with temperatures of between 22 and 26° C.

On the 1st day after the treatment, the weather was also sunny, warm and dry, with temperatures of around 25° C.

On the 2nd day, the sky was at first overcast with the temperature at 20° C, later the weather became less cloudy (temperature of 22° C), and continued to be dry.

It was not until towards the end of the 3rd day after the spray application that rain began to fall.

Thus the weather that prevailed after the treatment was also favourable for a successful control.

2. Test results

The test was carried out in practical conditions, using a Bell helicopter. The plots were marked out with balloons, and communication with the take-off area was established by radiotelephone.

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The test was evaluated by the following methods:

- a) Determination of knock-down by the trap sheet method.
- b) Determination of knock-down on marked-out areas.
- c) Observations of colonies.
- d) Sample fellings.

a) Determination of knock-down by the trap sheet method

Traps sheets measuring 1×1 metre were placed 30 centimetres above the ground at comparable points in each test plot, and a count was taken of knock-down at regular intervals. As only the different larval stages of pine sawfly — with the exception of stage VI — were present at the time of the test, percentage knock-down of larvae was recorded separately for each age group (stage).

The trap sheet counts for all larval stages together are given in Table 1.

Table 1: Results of trap sheet counts
(all larval stages together)

Products	No. of trap sheets evaluated	Average knock-down per trap sheet (1 m^2) in given no. of hours after treatment							
		4h	8h	12h	24h	36h	48h	60h	84h
DIPTEREX 40 l*	2	11	18	23	35	40	42	45	49
DIPTEREX 15 l	3	4	16	19	31	37	38	43	46
Comparison product	3	8	13	14	17	18	20	22	23

Mortality was most considerable in the DIPTEREX 40 l plot only four hours after the treatment whereas it was not high in the DIPTEREX 15 l plot. Knock-down started in the plot treated with the comparison product also after only 4 hours.

A striking feature of the results recorded is that the greater part of the advanced larval stages were knocked down in the first 12 hours after the treatment whereas knock-down of the younger stages (I, II) did not reach its peak until 24 hours after the application. This is accounted for by the habits of the larvae, and also provides an explanation for the low initial toxicity of the DIPTEREX 15 l/ha variant. The older larvae, although they behave socially, feed singly — 1 to 2 larvae per needle — whereas the young larvae, likewise, social, gather in tiers of 3 to 5 or even more on the needles. Thus, the older larvae can be hit on all sides by the spray solution whereas among the young stages only those larvae at the edge of the tiered cluster are wetted. The young larvae are not killed until they take up the spray deposit when feeding. Therefore, the insecticide acts more as a contact poison on the older larvae and as a stomach poison on the young stages. This explains why the older larvae, directly hit by the spray solution, are knocked down earlier than the majority of young larvae which first come into "contact" with the insecticide when feeding. The poorer initial toxicity of DIPTEREX applied at low volume (in 15 litres of water per hectare) is thus also comprehensible. But about:

* In all tables and references in the text:

DIPTEREX 40 l = 1.5 litres of DIPTEREX per hectare in 40 litres of water/ha.

DIPTEREX 15 l = 1.5 litres of DIPTEREX/ha in 15 litres of water/ha.



Fig. 1: Colonies of advanced larval stages (III—V) of pine sawfly feeding on young pine branches

hours after the treatment, there is practically no difference between the results of the two DIPTEREX variants, whether applied at low or high volume. In fact, the peak of knock-down produced by DIPTEREX at both volumes (40 and 15 litres of water per hectare) was reached 24 hours after the application, and was always about twice as high as the figure registered for the comparison product. Knock-down continued until the sample fellings were carried out. It should be mentioned that the majority of the larvae knocked down by DIPTEREX were found dead, whereas about a third were still alive in the plot treated with the comparison product.

b) Determination of knock-down on marked-out areas

In the order to check the results obtained by the trap sheet method, areas of 40 square metres each (2 × 20 metres) were marked out on the sandy paths running through the plots, and knock-down was counted in these marked-out areas 30 hours after the treatment. The results are given in Table 2.

As the initial infestation could not be established either in this method b) or in the trap sheet method, and a fairly useful standard of comparison was provided only by the density and the feeding damage, the findings outlined in a) and b) can only be of an orienting character.

Table 2: Knock-down on a ground area of 40 sq. metres per variant

Variant	No. of knocked down larvae
DIPTEREX 40 l	163
DIPTEREX 15 l	231
Comparison product	109 (approx. $\frac{1}{3}$ still alive)

This finding confirms the results of the trap-sheet count according to which there is no difference in effect between a volume of 40 litres per hectare and one of 15 litres per hectare.

c) Observations of colonies

Whereas the methods so far described did not enable the infestation to be determined, it was possible in the DIPTEREX 40 l plot and in the comparison product plot — in young cultures of only 1 to 3 metres high — to mark off colonies of the same larval stages, to count them before the treatment and to register the achieved knock-down. The results are given in Table 3.

Table 3: Knock-down in differently treated colonies of larvae
1. Larval stages II—III

Initial infestation	1.5 l DIPTEREX/ha in 40 l water/ha 106 larvae	Comparison product 99 larvae
after 4 hrs.	98	99
after 8 hrs.	63	98
after 12 hrs.	63	83
after 24 hrs.	31 some inactive	86
after 36 hrs.	31 all inactive	83
after 48 hrs.	24 all inactive	89

2. Larval stages IV—V

Initial infestation	1.5 l DIPTEREX/ha in 40 l water/ha 32 larvae	Comparison product 17 larvae
after 4 hrs.	28	16
after 8 hrs.	15	16
after 12 hrs.	9	23
after 24 hrs.	1	merged with
after 36 hrs.	0	other
after 48 hrs.	0	colonies

This result clearly shows the indisputable superiority of DIPTEREX over the comparison product.

Apart from these accurately counted colonies, 10 other colonies were marked off for each variant, and knock-down was registered, with the following findings:

Time of observation	DIPTEREX	Comparison product
4 hrs. after treatment	Excitation	Excitation
8 hrs. after treatment	Excitation 50 % decimation	Quietly feeding, no evident decimation
12 hrs. after treatment	Excitation 70 % decimation	as after 8 hrs.
24 hrs. after treatment	100 % decimation in stages III—V and 70 % in stages I—II with majority dead and glued to needles	Quietly feeding, no perceptible decimation, colonies continue to move about feeding and intermingle making an exact count impossible
36 hrs. after treatment	only 5—10 % of stages I—II glued to needles react to touch, all large stages knocked down	as above; isolated mortality in stages I—II but of no direct bearing on population density
48 hrs. after treatment	only very few individuals of stages II—III still alive	as above
60 hrs. after treatment	all dead	as above

It is evident from these results that the population in the DIPTEREX plot was so greatly decimated 36 hours after the treatment that for practical purposes the control could be rated as most successful already at this stage. An interesting feature is that about $\frac{1}{3}$ to $\frac{1}{4}$ of the younger larval stages remained glued to the needles when dead. The trap sheet method, therefore, does not provide a true picture of the situation in this particular case. Considering that in our case far more young than old larval stages were nevertheless found on the sheets, this leads to the conclusion that the control was carried out shortly after the larvae emerged from the eggs, which was later confirmed by the sample fellings.

d) Sample fellings

Four days after the treatment, 3 trees were felled close to the trap sheets in each plot, and immediately examined for infestation. It was observed that all the egg pods (10 to 30 per tree) had hatched out; for 100 eggs per pod and 50 % mortality, this means a population density of 500 to 1500 larvae per tree. We nearly always succeeded in finding the feeding nest for each pod.

Only dead larvae of stages I to III were found in the DIPTEREX plots (both volumes — 15 and 40 litres of water per hectare). Not a single live larva could be seen anywhere!

Practically no mortality at all was registered on pines sprayed with the comparison product and felled 4 days after the treatment.

This result fully corresponds with the observations and counts of the colonies. DIPTEREX applied at 1.5 litres per hectare in 40 or 15 litres of water per hectare gives 100 % control.

It should be mentioned that severe infestation of alder-trees by alder leaf beetle larvae was completely eliminated by both DIPTEREX and the comparison product. Only very few of the pine beauty moth caterpillars present in scattered parts of the DIPTEREX plots were still alive 4 days after the treatment. Thus DIPTEREX proved to be fully effective against all larval stages of pine sawfly and all larval stages of alder leaf beetle but did not give complete control of older larvae of pine beauty moth.

3. Discussion of results

The trial controls of pine sawfly carried out in the forest under favourable conditions, using DIPTEREX sprayed at 1.5 litres per hectare (50 % active ingredient) in volumes of 40 and 15 litres of water per hectare from a helicopter were completely successful.

According to the results of these tests, it is sufficient to spray DIPTEREX at low volume, i.e. at a dosage of 1.5 litres per hectare (50 % active ingredient) (corresponding to 750 g of active ingredient per hectare) in 15 litres of water per hectare.

A water volume of 40 litres per hectare is not necessary.

Despite these results, attention should be drawn to the following points. The test was carried out in the early morning when there was little wind, at a temperature of 18°C and a relative humidity of approximately 70 %. The application technique was excellent thanks to the outstanding way in which the pilot Baur flew and handled his helicopter. Losses by evaporation were thus reduced to a minimum. Under less favourable weather and application conditions (lower relative humidity, stronger wind, higher temperatures, poor distribution of spray solution as a result of flying too high), there is the risk that a volume of 15 litres of water per hectare will not be adequate. Further, more densely growing and taller stands may also make it necessary to use a higher volume. As we had normally developed stands in our test, a volume of 15 litres of water per hectare may be regarded as an average value.

4. Summary

The trial control of pine sawfly larvae in Central Franconia produced the following results:

1. Tests with DIPTEREX (50 % active ingredient), sprayed from a helicopter led to complete eradication of the pest.
2. The initial toxicity (cleanup) of DIPTEREX applied at a volume of 15 litres of water/ha is slower than when sprayed at a volume of 40 litres of water/ha.
3. There is practically no difference between the 2 DIPTEREX volumes with respect to knock-down 24 hours after the treatment.
4. All larvae of every stage had been exterminated in the DIPTEREX plots within 24 to 36 hours after the application.
5. Full control was also given of alder leaf beetle larvae.